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Fine-scale monitoring of running buffalo clover (Trifolium stoloniferum) restoration populations at Taylor Fork Ecological Area

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ABSTRACT

Fine-scale monitoring of running buffalo clover *(Trifolium stoloniferum)* restoration populations at Taylor Fork Ecological Area

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Abstract: Running buffalo clover (*Trifolium stoloniferum* Muhl. ex A. Eaton) is a species of clover that is federally listed as endangered. *Trifolium stoloniferum* requires mesic habitats with partially filtered light and will be outcompeted without periodic disturbance, such as grazing, mowing, or trampling. The purpose of this study was to understand rates of flowering and clonal reproduction associated with different growth stages of *T. stoloniferum*. During this study I visited 6 restoration sites of *T. stoloniferum* once each week from April to October, marking new individuals with a unique numbered metal tag and assessing the growth stage of individuals. All sites were assessed based on their stage structure, inflorescence production, clonal reproduction, and population growth. I hypothesized that populations with filtered light, reduced plant competition, and near disturbances such as streams and cow grazing, would perform better than populations lacking these conditions. Site two, which had disturbance caused by cows, was located near a stream, and had filtered light, had the highest percent of inflorescence production, highest percent of clonal reproduction, and highest population growth rate out of all 6 sites. Overall, all 6 sites grew in population size and produced new individuals, showing that these restoration populations were successful this season. For most populations, new individuals (ramets) started to appear near the end of July and the beginning of August.

These results support previous findings and are an important contribution to the restoration efforts made by researchers at Eastern Kentucky University and all over the nation.

Keywords and phrases: *running buffalo clover, Trifolium stoloniferum, disturbance, endangered species, population ecology, conservation, restoration*

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Introduction

Trifolium stoloniferum (running buffalo clover) was determined to be an endangered species by the federal government in 1987 (USFWS 2007) and so researchers are studying these plants to find ways to conserve the species. *Trifolium stoloniferum* is a perennial, stoloniferous species of clover that is a member of the Fabaceae, or pea, family. *Trifolium stoloniferum* was once thought to be extinct, but then was rediscovered in 1983 (USFWS 2007). *Trifolium stoloniferum* in Kentucky is mostly located in the central region (Figure 1) with the largest number of populations located at the Bluegrass Army Depot in Richmond, Kentucky (USFWS 2007). Populations of *T. stoloniferum* also occur in Ohio, Indiana, West Virginia, and Missouri.

Due to land use changes and factors such as the loss of bison and other large herbivores that cause disturbance, populations of this plant have decreased (USFWS 2007). Disturbance, from an ecological standpoint, is an event of environmental change that occurs over a short period of time, but causes pronounced changes to the ecosystem (Gurevitch et al. 2002). Some factors that led to *T. stoloniferum*'s decline could have been caused by humans, for example, building roads and cities, while others could have occurred naturally. There were reported associations with Native American trails and populations of *T. stoloniferum*, so the disturbance caused by these trails could have created or maintained premium habitats for *T. stoloniferum* (Campbell et al. 1988). The disappearance of these trails could have been an additional factor that led to their decline. Additionally, Native American management practices, for example setting intentional fires, were also associated with *T. stoloniferum's* success and the decrease in these prescribed fires could have also played a role in the population's decline (Burkhart 2010).

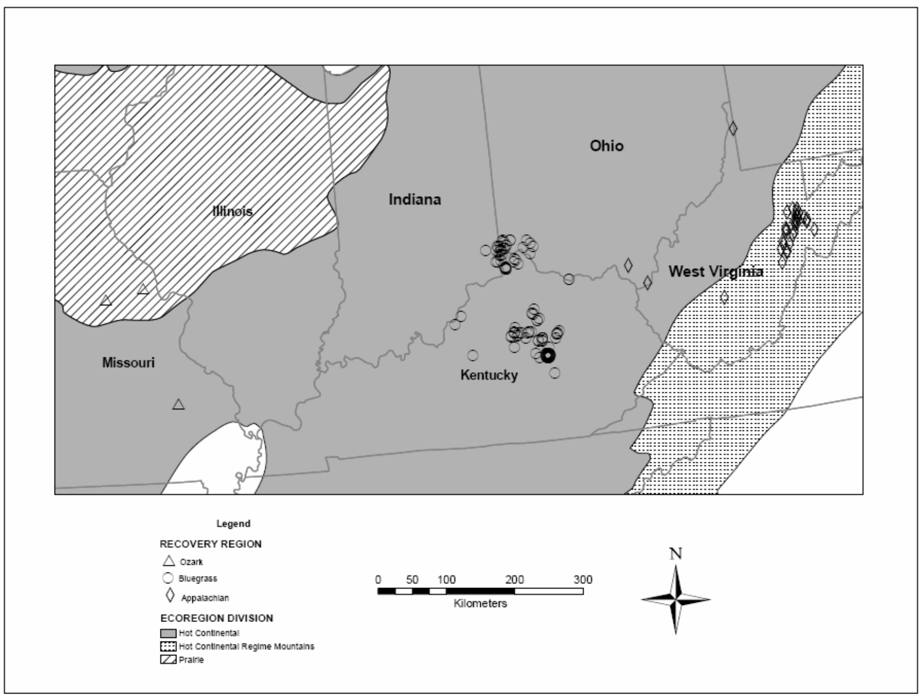

Figure 1: Map showing locations of *T. stoloniferum* provided by the United States Fish and Wildlife Service (2007).

Not many studies have been done on *T. stoloniferum*, but the ones that have been done have a common theme: disturbance. Many studies share results of disturbance helping populations of *T. stoloniferum* to increase in numbers. One study found that logging in the Fernow Experimental Forest contributed to *T. stoloniferum* population growth (Burkhart et al. 2013). Another study showed that a regular mowing schedule at Shawnee Lookout Park contributed to the growth of their *T. stoloniferum* populations (Becus and Klein 2002). The logging and the mowing schedule were both types of disturbances that could have increased population growth since they could have mimicked the disturbance of bison. One way disturbance plays an important role in the survival of *T. stoloniferum* is whenever herbivores are grazing they tend to pick off parts of the plants that are closest to them, so plants with stems growing upwards will typically have their stems eaten. Whenever plants stems are eaten their nodes are also eaten, which is where primary growth occurs. It will now take a long time for the plant to grow back to the state at which it was. With *T. stoloniferum*, their stems are stolons, which are low and parallel to the ground. Typically herbivores will only eat *T. stoloniferum*'s leaves so their stolons with the nodes are still intact. The stoloniferous growth form is likely an advantage in areas of herbivory compared to plants with an upright habit. Mowing can mimic what herbivores may do to the landscape, cutting down tall weeds and *T. stoloniferum*'s leaves but leaving *T. stoloniferum*'s nodes intact. It is important to note that too much disturbance may be a hindrance to *T. stoloniferum* as it prefers areas with filtered light (Hattenback 1996) and severe disturbance would remove the trees and other plants that provide partial shade.

A report from the Missouri Department of Conservation suggested that a reduced number of fires could have also been a reason for the decline in *T. stoloniferum* populations. Fires can create open fields where *T. stoloniferum* can grow and not be crowded out by existing plants and also cause scarification on the seeds of *T. stoloniferum* (Missouri Department of Conservation 2000). Scarification could also come from animals' digestive tracks (Watt 2011). Scarification helps break open seeds so they can germinate.

Trifolium stoloniferum does not have any chemical defenses against herbivores, which may be a reason why they are sought after by herbivores (Jacobs 1987). During a study at the University of Kentucky, their greenhouse population of *T. stoloniferum* succumbed to a viral or virus-like disease that was possibly transmitted from white clover (*Trifolium repens*, Jacobs 1987). Being susceptible to disease could have also led to the decline of *T. stoloniferum*.

A study was conducted to determine how genetically diverse populations of *T. stoloniferum* were and it was found that larger populations of *T. stoloniferum* had greater genetic diversity than smaller populations (Crawford and Windus 1995). This study shows how larger populations of *T. stoloniferum* will be more beneficial for the conservation of the species than smaller populations. A greater genetic diversity lessens the chance of inbreeding, which can allow expression of mutations that can be detrimental to the population.

One characteristic of *T. stoloniferum* that could affect its success is that the species does not go through the process of nitrogen fixation (Morris et. al. 2002). This means that the plant does not receive nitrogen through this specific process and has to rely on other methods, such as gaining nitrogen through the soil, to obtain this important element.

Factors Affecting *T. stoloniferum* Population Growth

Both sexual and asexual reproduction affect the population growth of *T. stoloniferum*. Some individuals will arise because of sexual reproduction (genets) and others by asexual reproduction (ramets). Whenever *T. stoloniferum* reproduces asexually, the parent plant produces stolons that new genetically identical individuals can grow from. Sexual reproduction is carried out by flowering and individuals growing from seeds will not be connected to another ramet.

A plant's size can have a great effect on its reproductive capabilities (Gurevitch et al. 2002). Differentiation in plant stages is therefore an important component in determining how successful a plant can be. *Trifolium stoloniferum* has 6 different stages that it can be classified into (Hickey 1995). These different stages can affect population growth because *T. stoloniferum* is reproductive at higher stages when they have stolons and flowers. Also, larger individuals that have been around for a few years will likely be more productive than smaller, newly planted individuals (Gurevitch et al. 2002).

Objectives and Hypotheses

During this study I monitored 6 restoration populations of *T. stoloniferum* at Taylor Fork Ecological Area and the adjacent Tudor Farm for an entire growing season. I counted and tracked *T. stoloniferum* to determine how plants are transitioning between stages, the number of plants producing flowers, and clonal reproduction by marking new crowns as they separated from their parental crown. I also determined how these stage transitions and general biotic and abiotic conditions affected population growth. Based on previous studies in other locations around the country, I hypothesized that *T. stoloniferum* in areas with filtered light, disturbance, and further away from competitive vegetation would be more productive in terms of both clonal and sexual reproduction than *T. stoloniferum* not in those areas. I also hypothesized that the stages would have an effect on population growth, with an increase in population growth after plants reached stage three since this is the smallest stage with stolons. This study was conducted to add to the research that has been previously conducted at Eastern Kentucky University to learn more about the populations we have here in Richmond, Kentucky and the species overall.

Methods

Study Species

Trifolium stoloniferum can be recognized by its paired leaves below the inflorescences and stolons branching out along the ground from the crown stem (Figure 2). A crown stem is defined as rosette that is rooted into the ground (USFWS 2007). Flowering occurs from mid-April to June while fruiting occurs from May to July (USFWS 2007). *Trifolium stoloniferum* lacks the white stripes down the center of the leaflet and hairs on the stems and leaves that are commonly seen in white clover (*Trifolium repens*). *Trifolium stoloniferum* plants also have toothed edges around the leaflets, which are similar to other common clovers (Burkhart 2010). *Trifolium stoloniferum* has a prominent stipule at the base of the leaves that is absent in white clover (USFWS 2007).

Figure 2: Illustration of *T. stoloniferum* depicting characteristics (Burkhart 2010).

Like other clovers, *T. stoloniferum* produces inflorescences. Inflorescences are the whole flower head of *T. stoloniferum* (and other plants) that includes the stems, stalks, bracts, and flowers. *Trifolium stoloniferum* has multiple flowers on one head. What we may conventionally think is one flower is actually multiple individual flowers and what we may think is a petal is actually a single flower (Figure 3).

Figure 3: This image shows a *T. stoloniferum* inflorescence (Burkhart 2010).

E. Hickey proposed that there are a total of 6 stages in the growth of *T. stoloniferum* (Hickey 1995, Figure 4). Local researchers have formalized these definitions as described below (Dart-Padover et al. 2014).

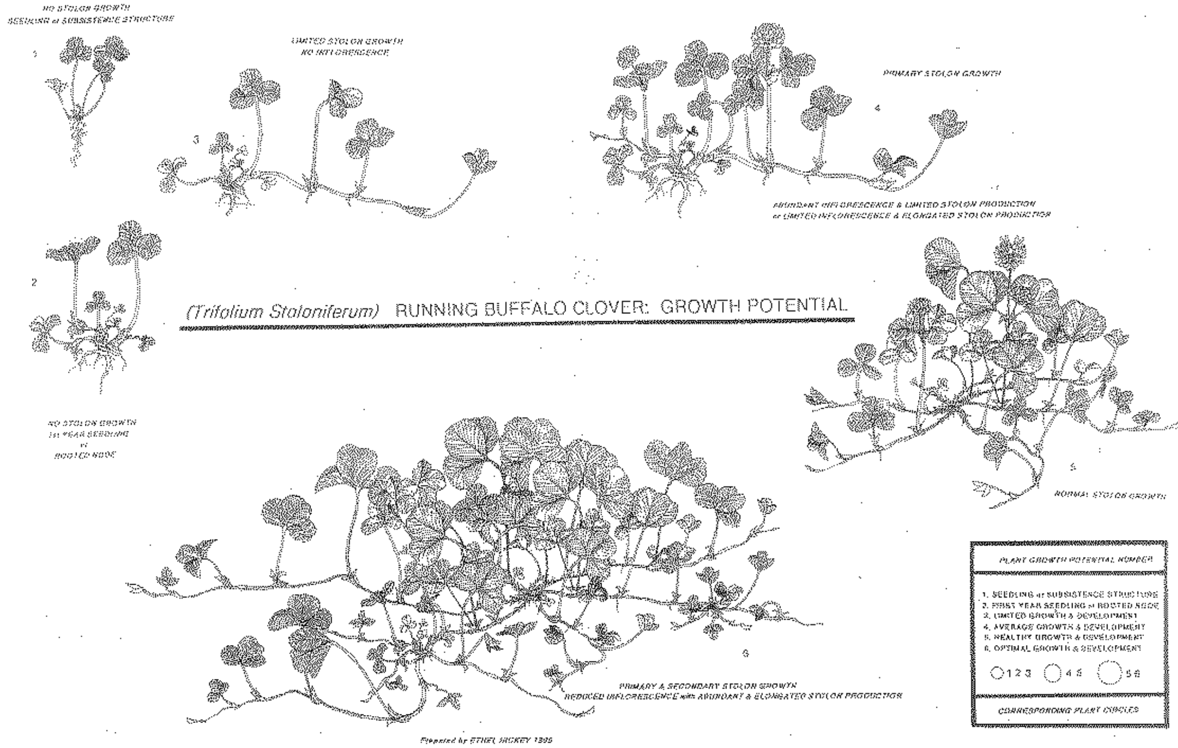

Figure 4: E. Hickey's drawings and descriptions of the 6 stages of *T. stoloniferum*.

The first stage is a seedling stage that may have a few short leaves. The second stage has no stolons or inflorescences. The third stage has 1 stolon or a total stolon length that is less than 50cm while still not having any inflorescences. Plants in stage three tend to go through limited growth and development. Plants in stage four have 1 to 3 inflorescences or no inflorescences, but stage four plants must have 2 or more stolons with a total length greater than 50cm. Plants in this stage have an average amount of growth and development occurring. The fifth stage has more than 4 inflorescences or has no inflorescences, but 4 or more stolons with a total length greater than 100cm. These plants are noted for their healthy growth and development. The sixth stage has new crown stems forming and rooting and/or stolons forking. *Trifolium stoloniferum* that are in stage six are going through an optimal amount of growth and development.

Study Sites

There are a total of 7 *T. stoloniferum* populations at Taylor Fork Ecological area and the adjacent Tudor Farm. Both of these areas are located on the campus of Eastern Kentucky University in Richmond, Kentucky (Figure 5). Only 6 of these populations were observed for this study. Sites two, four, and five were previously assessed in the Fall of 2014 (Pauley and Koslow 2014).

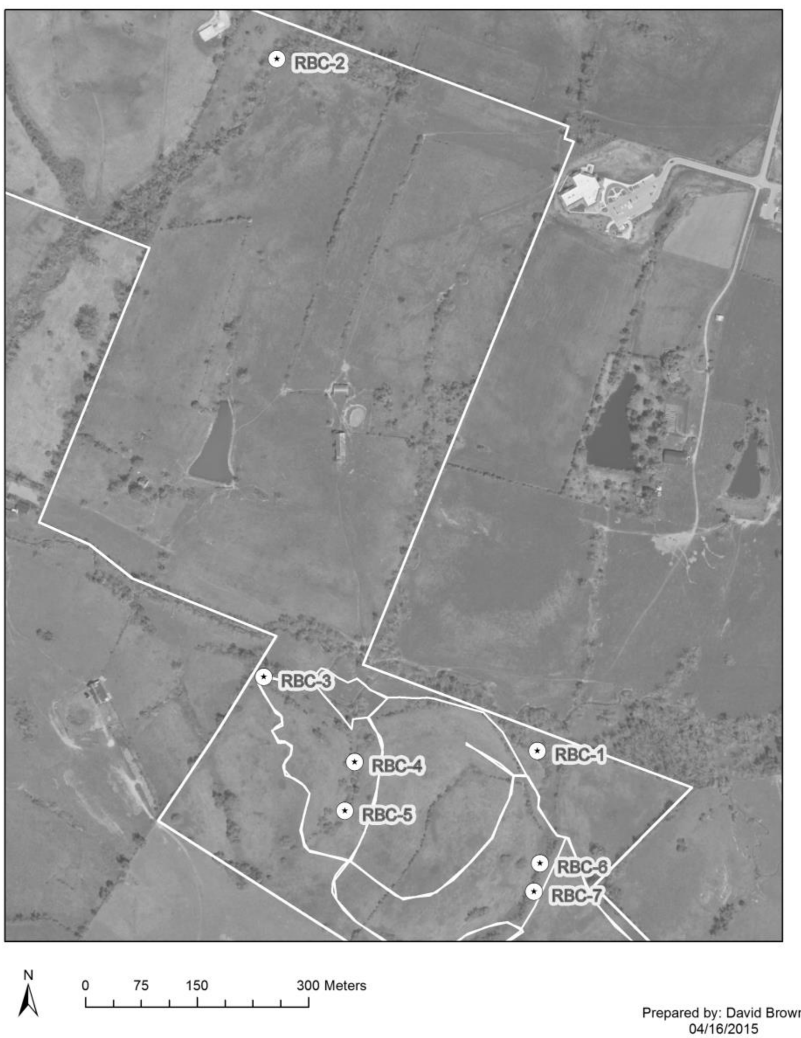

Figure 5: Locations of *T. stoloniferum* restoration populations at Taylor Fork Ecological Area and the adjacent Tudor Farm on the campus of Eastern Kentucky University in Richmond, Kentucky. Sites are indicated by “RBC” followed by their site number. By Dr. David Brown

Site one was an experimental population that was not observed in this study as it was used for research on herbicide treatments. Site two, located at Tudor Farm, is the oldest restoration population and had the greatest number of individuals. This site had 1 *T. stoloniferum* individual planted there in 2012 and 12 individuals planted there in 2013. The final count last fall had 142 individuals. Due to the large number of individuals at the site, a sub-plot was created and was monitored every other week along with the other 5 sites. During the week when the 6 sites weren’t measured, data were collected on the other individuals that were situated in site two (excluding the sub-plot plants). Site two (Figure 6) had the best conditions for *T. stoloniferum,* which included cow disturbance, filtered light, and close proximity to a stream. This stream attracted cows and other wildlife that caused disturbance. In addition, during times of high rain the stream can flood and deposit nutrients into the soil.

Figure 6: Photo of site two at Tudor Farm taken by Chelsea Perkins

Site three had an unknown number of individuals planted there in 2012. There is a high possibility that several of these 2012 plants have died. In 2014 more individuals were planted. Site three had the second best conditions, having filtered light and being near a trail that had occasional mowing. Site four had 9 individuals and site five had 11 individuals in November 2014. Site four was situated just off a trail that received occasional mowing and was situated in high sun, but with tall weeds and young trees/saplings that provided some during shade the day, depending on position of the sun. Site five was located in a secluded location off the same trail as site four, but set back further from the trail. This site was in a shaded area with evidence of a stream nearby, which was dry most of the season, if not all. Sites six and seven are smaller populations that were planted in the summer of 2014 and both were situated along a small stream. Sites six and seven had by far the worst conditions of the 6 sites that were studied (Figure

7). Both sites were in heavy shade with a dense plant community surrounding them. They were also located far off the same path, but through thick weeds.

Figure 7: Site seven's location at Taylor Fork Ecological Area. These plants are located within the trees pictured here.

Field data collection

I made a trip to Taylor Fork and Tudor Farm once each week from April to October, marking new individuals and assessing the growth stage of individuals at each site. New individuals were marked with a unique numbered metal tag placed between the plant and a marked reference tree for that population. In the case of site two, tags were placed between the plant and a bridge near the population. Tag locations were standardized so future observers would know which tag goes with which individual. All data referencing site two are taken from the sub-plot that was placed within the site. Although data collection at the large population at site two started in April, standardized data collection on the 6 sites (including the sub-plot) did not start until June 7th, 2015 and continued through October 24th, 2015.

When marking individuals I determined which stage they were in, number of stolons, stolon length, number of crown stems, and number of inflorescences. When new plants were discovered, the origin of the plant along with a confidence value was also recorded. The origin of the plant simply means which parent plant this new, genetically identical individual is coming from. The confidence value was a number from one to three to indicate how confident I am in where they originated from. For this study a lower confidence was one while a higher confidence was three, with two being in between. Only asexual reproduction affected the population growth for the sites since fruits were collected at all 6 sites for use in other research in the middle of June in 2014 and 2015. I determined the number of plants in each stage for each sampling period and site, the percent of inflorescence production, and percent of clonal reproduction for each site. To determine *T. stoloniferum*'s clonal reproduction for each population I divided the number of genetically identical offspring the parent plants produced by the original number of individuals at the site and converted that number into a percent. To determine geometric population growth (λ), I used the formula $\lambda = \frac{N_{t+1}}{N_t}$. This is the ratio of population sizes at two different times, so N_{t+1} was the population size at the end of the season while N_t was the population at the beginning of the season. If λ is larger than 1, then the population is growing.

Results

Stage Structures

The sub-plot of site two had a total of 7 individuals at the beginning of the season and 35 individuals at the end of the season, meaning there was an increase in the population size (Table 1). One plant was discovered on June 19th that was there from last season that had accidentally been skipped over on June 7th, so that is why there were 7 original plants instead of 6. New individuals began to arise at the end of July and beginning of August with an increase in stage two individuals and a decrease in stage five and six individuals. At the beginning of the season I found 5 individuals that were dead. Later, toward the end of the season, more individuals died off.

Site Two Stage Structure

Stage	**7-Jun**	**19-Jun**	**26-Jun**	**8-Jul**	**23-Jul**	**7-Aug**	**24-Aug**	**6-Sep**	**30-Sep**	**21-Oct**
2	1				15	24	29	32	36	35
3		2	2	3	3	2	2	2		
4	3	3	3	3	3	4	2	1		
5	1	1	1	1						
6	1	1	1	1	2					
Dead	5					1	2	2	4	5
Alive	**6**	**7**	**7**	**7**	**23**	**30**	**33**	**35**	**36**	**35**

Table 1: Stage structure over an entire growing season for site two.

Site three started with 18 individuals and had a final population of 48 individuals, indicating population growth (Table 2). A number of new stage two individuals appeared at the beginning of August and continued to appear until the end of the season. Co-occurring with the appearance of new stage two individual were the disappearances of stage four and five individuals. Mortality appeared again near the end of the season, picking up at the end of September.

Site Three Stage Structure

Stage	7-Jun	19-Jun	26-Jun	8-Jul	22-Jul	7-Aug	24-Aug	6-Sep	30-Sep	24-Oct
2	4	4	4	4	4	14	23	28	36	47
3	5	5	5	6	4	5	6	5	2	1
4	4	5	5	4	3	2	1			
5	5	4	4	4	4	3				
6						2	1	1	1	
Dead					2	2	5	6	12	12
Alive	**18**	**18**	**18**	**18**	**15**	**26**	**31**	**34**	**39**	**48**

Table 2: Stage structure over an entire growing season for site three.

Site four had an original population size of 9 and ended with 37 *T. stoloniferum* individuals (Table 3). During the winter season 1 plant died as 1 individual was marked as dead the first time data was collected. New individuals appeared at the end of August, accompanied with an increase in stage two individuals and the disappearance of a stage three individual. Like the other sites, we again had mortality occurring at the end of the season.

Site Four Stage Structure

Stage	7-Jun	19-Jun	26-Jun	8-Jul	22-Jul	7-Aug	26-Aug	6-Sep	7-Oct	21-Oct
2	1	1	1	1	1	1	6	7	8	32
3	3	3	3	3	3	2	1		4	3
4	2	3	2	2	3	3	2	2		
5	3	2	3	3	2	2	2	2	1	
6							1	1		2
Dead	1					1	2	2	2	2
Alive	**9**	**9**	**9**	**9**	**9**	**9**	**12**	**12**	**13**	**37**

Table 3: Stage structure over an entire growing season for site four.

There was an increase in population size from 7 plants to 16 plants for site five (Table 4). There were no stage five or six plants at this site. Winter mortality was recorded the first time the plot was sampled but no individuals died during the season. New individuals for this plot did not appear until October, which is indicated by the increase in stage two plants and decrease in stage three plants.

Site Five Stage Structure

Stage	4-Jun	7-Jun	19-Jun	26-Jun	8-Jul	22-Jul	7-Aug	26-Aug	6-Sep	10-Oct	24-Oct
2	1	1	1	1	1	1	1	1	4	11	13
3	5	6	6	6	6	6	6	6	5	3	3
4	1										
Dead	4										
Alive	**7**	**7**	**7**	**7**	**7**	**7**	**7**	**7**	**9**	**14**	**16**

Table 4: Stage structure over an entire growing season for site five. The date October 10th is red because I was not able to finish getting data on that site on October 7th due to unforeseen circumstances. The rest of the plants were assessed on October 14th, so the date (October 10th) was just averaged and data were put together on one day.

For site six there was a double in population size, which started at 3 plants and increased to 6. New stage two individuals appeared at the beginning of August and at the same time the number of stage three individuals decreased (Table 5). For this site there were only stage two and stage three individuals and no stages larger than three appeared during the entire season. One individual died at this site the same time new stage two individuals appeared.

Site Six Stage Structure

Stage	7-Jun	19-Jun	26-Jun	9-Jul	22-Jul	7-Aug	24-Aug	6-Sep	7-Oct	21-Oct
2						3	3	4	4	5
3	3	3	3	3	3	2	2	1	1	1
Dead						1	1	1	1	1
Alive	**3**	**3**	**3**	**3**	**3**	**5**	**5**	**5**	**5**	**6**

Table 5: Stage structure over an entire growing season for site six.

For site seven there was a double in population size, like site four, with an increase from 4 to 8 plants (Table 6). There was no mortality at this site. Stage two individuals did not increase until the end of October.

Site Seven Stage Structure

Stage	7-Jun	19-Jun	26-Jun	9-Jul	22-Jul	7-Aug	24-Aug	6-Sep	7-Oct	21-Oct
2	2	2	2	2	2	2	2	2	2	6
3	1	1	1	1	1	2	2	2	2	2
4	1	1	1	1	1					
Alive	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**8**

Table 6: Stage structure over an entire growing season for site seven.

Inflorescence Production

Site two had the greatest percent of inflorescence production, while sites three and five followed behind (Figure 8). This means that about half (57. 14%) of the original 7 plants that were at site two produced inflorescences. Only 11.11% of site three's original 18 plants produced any inflorescences and 14.29% of site five's 7 plants produced inflorescences. Sites four, six, and seven had no inflorescence production occurring.

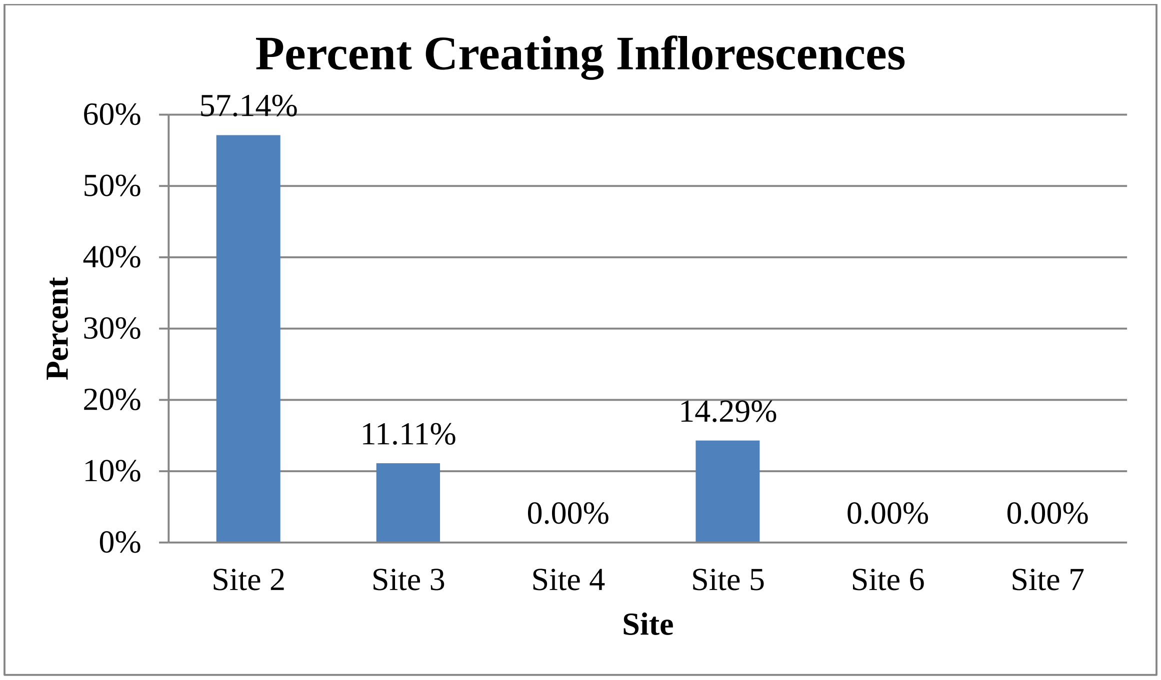

Figure 8: A graph comparing inflorescence production at all 6 sites.

Clonal Reproduction

Site two was the most successful in clonal reproduction, with 100% of the plants producing a new ramet (Figure 9). Site four did the next best, with 88.89% of the original individuals producing offspring. Site five was close in clonal reproduction with site four by having 85.71% of the original plants producing new individuals. Both sites three and six had 66.67% percent reproduction and site seven did the worst with only 25% clonal reproduction.

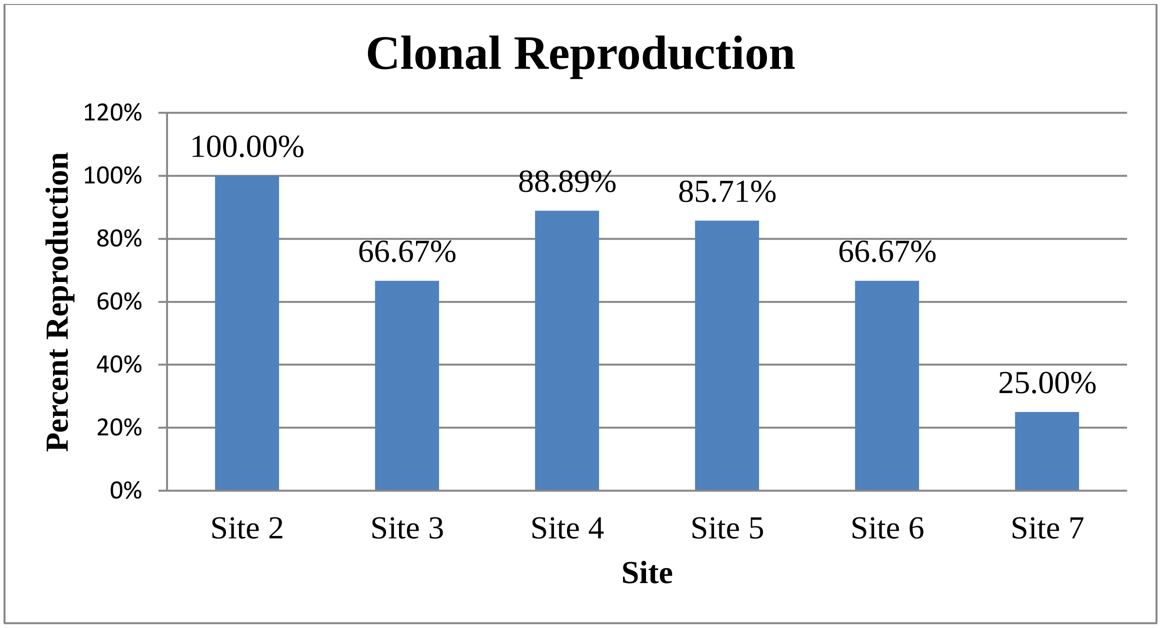

Figure 9: This graph shows a comparison of clonal reproduction of all 6 sites.

Population Growth

Site two's *T. stoloniferum* population increased by 5 times (Figure 10). Site four did the next best by increasing in population size by 4.11 times. Site three's population size increased by 2.76 times and site five increased by 2.29 times. Both sites six and seven only increased in population size by 2 times. Overall, we see that all 6 populations increased in size since all of their values were greater than 1.

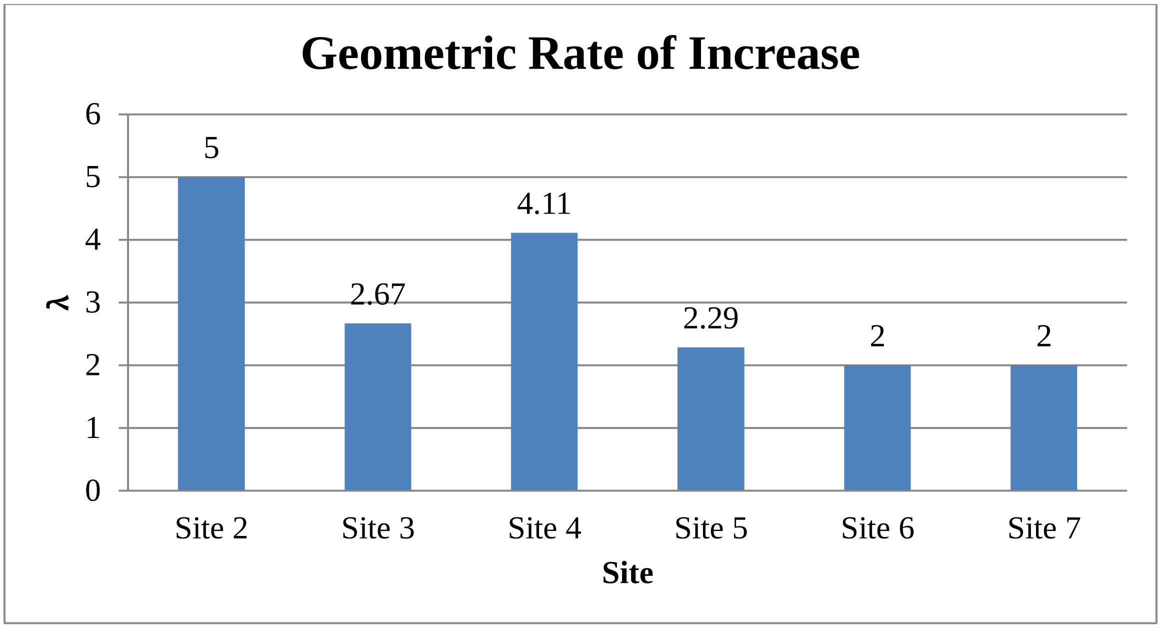

Figure 10: A graph showing the geometric rate of increase (λ) for each site.

Discussion

All populations of *T. stoloniferum* at Taylor Fork and Tudor Farm were growing through clonal reproduction and half of the sites produced flowers. In most populations clonal reproduction started to occur near the end of July and the beginning of August while flowering began around May. For several populations, there were plants that were dead from the beginning of data collection. These were plants that suffered mortality during the winter. Also near the end of this season, mortality was seen once again and it is expected that some surviving plants from this season will die over the winter.

Stage Structures

In all 6 sites there were new *T. stoloniferum* individuals arising in the populations around the end of July or beginning of August. New individuals were usually in stage two, and as these new plants separated from the parent plant, the parent plant tended to regress to a smaller stage. This regression happened because whenever a new crown stem rooted and detached from the stolons of its parent plant, the parent plant decreased in the number of stolons it had, generally causing it to be classified as a smaller stage. Sites five, six, and seven did not have any stage five or stage six individuals this season while the other 3 sites did. These sites lacked these higher staged individuals because of their location. These 3 sites were in some of the worst conditions, with heavy shade and less disturbance.

Inflorescence Production

Site two has the greatest percent of inflorescence production among the 6 sites. Out of the original 7 plants that were in site two, 4 plants produced inflorescences. Site two had the most flowering due to its location and the age of the plants there, as it is the oldest site compared to the other 5 sites. Again, this site has the best conditions for *T. stoloniferum* to grow in out of the 6 sites that were studied (filtered light, cow disturbance, and a nearby stream). Site five did the next best with 1 out of 7 original plants flowering and site three followed behind with only 2 out of 18 original *T. stoloniferum* plants producing inflorescences. Since these percentages are relative to the size of the population, site five did better than site three, even though site three had 2 instead of 1 flowering individuals, because site five had a smaller original population. Site three is the second best site when considering conditions (filtered light and near a trail with mowing), so flower production is expected. Since site three also had an unknown number of individuals planted there in 2012, these older individuals may have increased site three’s success as well. Site five was one of the sites with the worst conditions, being in a site far off a trail and in shaded light, but it was by far in better conditions than sites six and seven.

Clonal Reproduction

Out of all 6 sites at Taylor Fork, site two had the greatest amount of clonal reproduction. All 7 original plants produced at least 1 new ramet due to their ideal location. Site four did the next best with 8 out of 9 plants producing at least 1 ramet. Site five had 6 out of its original 7 plants producing ramets. About 67% of plants at both sites three and six underwent clonal reproduction. Site three had 12 out of 18 original plants clonally reproducing and site six had 2 out its original 3 reproducing asexually. Site seven did the worst due to its location, with only 1 out of its original 4 individuals producing any new offspring. Between sites six and seven, site seven was worse off than site six even though their locations were similar. Site seven was further from a stream and so was not able to benefit from potential wildlife or stream disturbance.

Population Growth

Site two increased its population size by a factor of 5 (Figure 10). This site started with only 7 individuals and ended with 35, increasing its population size by 28 individuals. Once again, its population growth is attributed to its location at Tudor Farm. The conditions that the site provided for *T. stoloniferum* were optimum for population growth and it is promising to see this population doing so well since it is a restoration population and not a natural population. Surprisingly enough site four followed behind site two's success with the population growing by a factor of about 4, increasing from 9 to 37 individuals. This is unexpected due to its location being surrounded by competition and in direct sunlight. I attribute the success of this population to the high growth rates of a few individuals that were growing under the partial shade of some young trees. Site three's population increased by a factor of about 3. It increased from 18 individuals to 48, so an increase of 30 individuals. Sites five, six, and seven did about the same, increasing by about a factor of 2. All 3 of these sites had poorer conditions, especially sites six and seven that led them to just surviving and producing a few new individuals.

Population Success

A question one may ask is why did the populations of *T. stoloniferum* do so well this season? One possible answer to this is the weather we had this season. At the beginning of *T. stoloniferum*'s growing season there was plenty of steady rain, so these individuals had little drought stress. This could be an important factor as to why all the populations increased and produced new ramets. Near the end of the season some plants started to die, co-occurring with a small drought the area was having, which could have led to their decline near the end of the growing season.

Recommendations

For future sampling I recommend continuing to visit all the sites every other week rather than every week. By visiting the sites every other week you will still see the important changes. At times during the season, not much changes in the size of the plants, so nothing will be missed by going every other week. I also recommend obtaining data on plant composition and light conditions for each site. These data would help further support previous studies' claims that *T. stoloniferum* plants are negatively affected by competitors and that they prefer filtered light.

I also have a recommendation for a follow-up analysis. I would try to examine relationships between survival and production of crown stems. By observing the trends in the survival of the parent plants and their reproduction, there may be instances that a plant will have several offspring and then the parent plant would die that season or the next. In essence, there may be a trade-off between survival and reproduction. Another possibility is looking into the effects cows have on population growth. By placing two different populations in the same area, but restricting the cows' access to one, it would be possible to observe the relationship between cows and *T. stoloniferum*'s population growth. A study on the contribution between sexual reproduction (through seed germination) to population growth would also contribute to conservation efforts.

Conclusion

The general trend that we see with all the data that were collected was that out of all 6 sites located at Eastern Kentucky University, site two was the most successful and most promising site. This is also the oldest out of the 6, which could be a reason why it is so successful, but the conditions in which the site is located also is something to consider when thinking of its success. This site has cows that frequently visit (Figure 11). Cows cause disturbance that reduces competition and also provide nutrients to the soil with their droppings. The trees that surround the site cover the area just enough to allow filtered light to reach the ground, which is what *T. stoloniferum* prefers rather than direct sunlight or heavy shade. Even though this site did have many taller grasses and weeds surrounding the plants, the cow's disturbance, and even my own disturbance, reduced some amount of competition. The stream provides even further positive impacts by attracting wildlife and depositing nutrients into the soil during high flood events.

Previous literature stated that areas with disturbance would have greater *T. stoloniferum* production, and so my findings during this study support the literature. Also, my original hypothesis that *T. stoloniferum* populations with filtered light and near disturbance would do the best was correct. Sites two and three are good examples supporting my hypothesis. These data also support my hypothesis that if there were more individuals in either stage five or six in a population, then there was a greater reproductive output for that site.

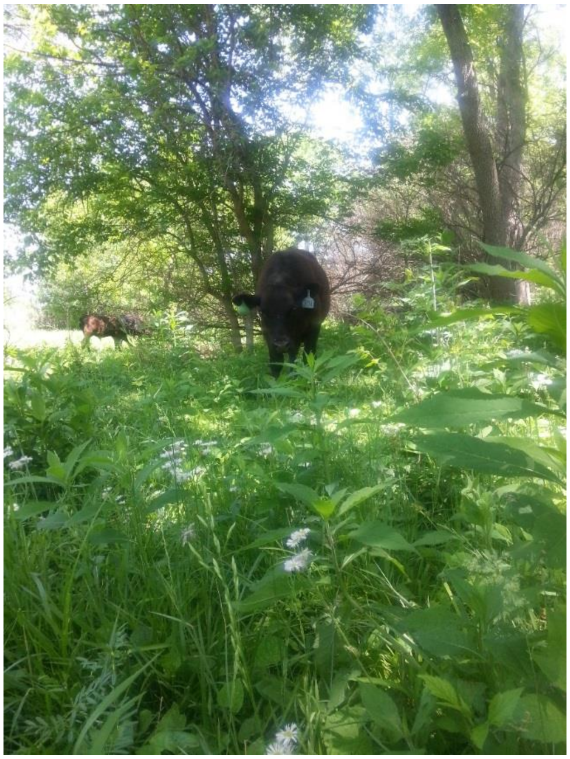

Figure 11: Picture of a cow at site two taken by Chelsea Perkins

This season every population at Taylor Fork was able to survive and reproduce. Not all populations were thriving, but the oldest population was the most successful. Next season we may see that these three newer populations, sites three, six, and seven, may do even better than they did this season since now they have more individuals. I predict that site three will be the one of these 3 newer populations to be successful next season, considering its location and that it was also a larger site to begin with this season.

This study shows that restoration populations of *T. stoloniferum* can survive and be successful. Restoration is a very important aspect of conservation biology and the more successful restoration populations we are able to produce, the greater the chances *T. stoloniferum* has of bouncing back from its endangered status. With greater understanding of *T. stoloniferum* and once 30 secure, self-sustaining populations exist, *T. stoloniferum* can possibly be reclassified as a threated species instead of an endangered species (USFWS 2007).

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